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**Organophosphorus Pesticide Poisonings in
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Organophosphorus Pesticide Poisonings in Humans: Determination of Residues and Metabolites in Tissues and Urine

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ABSTRACT

The analyses of four organophosphorus pesticide poisoning cases, three of which resulted in death, are reported. The case histories of the subjects, along with the analysis of tissues, urine, and blood for the levels of pesticides and metabolites are given. The pesticides involved include dicotophos, chlorpyrifos, malathion, and parathion. The methods of analysis were adapted from previously published methods that provide a very rapid means of identification of organophosphorus pesticides in the tissues or in the blood of poisoned patients.

RECENTLY DEVELOPED METHODS for the analysis of organophosphorus pesticide metabolites¹⁻³ have been limited in use to the determination of urinary metabolites. The need to identify toxicants in samples other than urine led to the expansion of these methods to include the analysis of blood, brain, stomach contents, and liver. It was found that the alkyl phosphate method allowed a simultaneous determination of intact pesticides and the alkyl phosphate metabolites. Application of these methods to the analysis of four cases of accidental human poisoning, along with the case histories and the tissue levels of various pesticides and their metabolites, are reported. When levels of the pesticide in tissue were sufficiently high, the identification of the toxicant was confirmed by gas chromatography-mass spectrometry (GC-MS). Pesticides that were found in these tissues include dicotophos, chlorpyrifos, malathion, and parathion. The need for a rapid means of identification of the type of organophosphorus pesticide involved in a poisoning incident is demonstrated in a recent article on fat-soluble organophosphate poisonings.⁴ By identifying the pesticide as one of the lipophilic types, the delayed onset of symptoms can be predicted and treated. This method can be used to provide a preliminary identification of the common organophosphorus pesticides very rapidly, using any of several biological samples. With the levels of pesticides encountered in poisoning incidents and the specificity of the detectors used for the analysis of organophosphorus pesticides, there are no interfering compounds to be removed by a cleanup step. The elimination of the cleanup steps and waiting periods necessary for quantitative analyses can cut the analysis time to as little as 30 minutes.

Methodology

The method used for the analysis for alkyl phosphates in urine was previously reported.¹ This method was expanded to include tissue by grinding a 0.5-g sample of tissue in an equal amount of distilled water in a Kontes glass pestle homogenizer (Catalog # K88545). The homogenate was then extracted with 10 ml of acetone, and centrifuged. A small portion (about 0.1 ml) of the acetone supernatant was reserved for the GC analysis of intact pesticides. The remaining supernatant was transferred to an ion exchange column and analysis for alkyl phosphate metabolites was continued as reported by Lores and Bradway.¹

The 0.1-ml aliquot of acetone extract, which was reserved for analysis of intact pesticides, was injected without cleanup, into a 5% OV 210 column coupled with a flame photometric detector being operated in the phosphorus mode. Any peaks obtained from this injection were compared to an ethyl parathion standard. For tentative identification of the intact pesticide, the relative retention time was compared to a chart of relative retention times.⁵ The sample was then compared to a standard of the suspected pesticide. If the level of pesticide in the tissue appeared to be high enough, an additional portion of the sample was extracted and prepared for GC-MS confirmation. In some cases the formulation containing the suspected toxicant was available and an extract of the formulation was subjected to GC-MS confirmation for the toxicant.

The phenol metabolite was analyzed using the multi-residue procedure of Shafik et al.³ This analysis was run on only two samples: the gastric contents from Patient II, and the urine from Patient IV.

Case Histories

The histories given here were obtained from the Office of the Chief Medical Examiner for the State of North Carolina, with the exception of that of Patient I, which was submitted by Dr. Wayland J. Hayes of Vanderbilt University Hospital.

Patient I

The patient was admitted to the hospital on March 11, 1975. While under the influence of alcohol he had apparently been drinking from a gallon jug labeled "turpentine." The jug was recovered with about three-fourths of the contents remaining. The moderately deep amber color of the contents indicated that something had been added to the turpentine. At admission, the patient was unconscious, but various signs suggested poisoning by an organophosphorus compound. His blood cholinesterase later proved to be zero. He remained in an unconscious condition for most of the first 10 days at the hospital. He responded, however, to repeated high doses of atropine and 2-PAM iodide. In spite of intense treatment, he continued to show some signs of poisoning, mainly sweating. He was later reported to be alert, eating well, and on the road to recovery.

Patient II

The subject, who had a history of overindulgence in both food and drink, died at home after being out drinking.

Patient III

At 2:00 P.M. on Saturday, August 23, 1975, a 13-year-old male American Indian was pronounced dead on arrival at the hospital. He was brought in by his parents, who reported that the boy had worked harvesting tobacco until about noon on that date. This death is attributable to organophosphate poisoning, resulting from dermal contact with parathion residue less than 24 hr after its application to the tobacco in which the boy had worked. The deceased had been in good general health and had worked regularly all summer at harvesting tobacco without becoming ill. He and his best friend had worked on this particular farm for five days preceding his death. On Friday, August 22, they were asked to help "finish the crop" on Saturday by picking the final leaves from the stalks.

The farmer directed one of his employees to apply an insecticide to the tobacco on that Friday afternoon. After learning the field had been sprayed, some of the workers expressed concern on Friday and again on Saturday morning. The farmer assured them it was "safe to work," but three workers reportedly refused to work that day and left.

The deceased began complaining of weakness and abdominal pain about 10:00 A.M., Saturday, but kept trying to work. He then became worse and left the field. After harvest was completed, he was taken home, arriving about 1:00 P.M. He immediately went inside to lie down. His par-

ents came home a short while later, found him unconscious and drooling from his mouth, and they took him to the hospital, where he was pronounced dead on arrival.

The best friend of the deceased began to experience dizziness, nausea, and vomiting during the afternoon of Saturday, the 23rd. His mother took him to the emergency room of the hospital, where, according to the mother, he was diagnosed as having a "grief reaction" because of his friend's death. Another co-worker returned home the afternoon of the 23rd and went to lie down, complaining of abdominal pain. His mother made him bathe immediately, and he had no further symptoms.

The applicator of the pesticide stated that, on the farmer's direction, the tobacco crop had been sprayed on Friday afternoon (August 22) with the contents from a "black metal can." He said he did not remember the name of the spray, admitted that he could not read, and that he disposed of the can at a landfill. The farmer also said that he did not recall what pesticide was used.

Patient IV

A 61-year-old white male with a history of heart disease, carcinoma of the tongue, and cirrhosis of the liver, accidentally ingested an unknown quantity of pesticide on January 12, 1976. The pesticide had been prepared by his

wife in a plastic milk bottle and marked with a label "Poison." Apparently she had been keeping the bottle at a neighbor's house and, on that day, had brought it home to use the pesticide. Her 11-year-old daughter saw what appeared to be a bottle of milk and placed it in the refrigerator. Shortly thereafter, the deceased removed the bottle from the refrigerator and took a drink, thinking it was milk. He immediately spit it out but was taken to a hospital, where he died approximately a day later. The cholinesterase level on blood drawn during his hospital admission showed a level of only 1 unit, compared to a normal level of 45 to 90 units.

Results and Discussion

The Table lists the results obtained from the analysis of all the samples from the poisoning incidents.

In Patient I, the only samples available were urine and the remainder of the ingested formulation. The toxicant was first identified as the dimethyl phosphate of 3-hydroxy-*N*-methyl-*cis*-crotonamide, or monocrotophos, but GC-MS indicated a compound with one additional methyl group. The compound was therefore identified as the dimethyl phosphate of 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide, or dicrotophos, which has the same retention time as monocrotophos on GC. The alkyl phosphate metabolite corre-

sponding to dicrotophos, *O, O*-dimethyl phosphate (here called DMP), was found in the urine sample at a level of 5 ppm.

In Patient II, the toxicant was not conclusively identified. It was believed, however, to be one of the pesticides that decompose to 2, 2-dichlorovinyl dimethyl phosphate,⁶ or dichlorovos, which subsequently hydrolyzed rapidly to DMP. Large amounts of DMP were found in the stomach contents, but a phenol analysis was negative. The lack of a phenol in the stomach contents implies an aliphatic side chain in the intact pesticide. A sample of the liver was the only one that showed any signs of an intact pesticide, but because the compound had apparently hydrolyzed, subsequent analysis precluded confirmation by GC-MS.

In Patient III, whole blood was the only sample submitted. Analysis for intact organophosphorus pesticides indicated a number of minor peaks and two larger peaks. One of the larger peaks had a retention time identical to that of the ethyl parathion reference standard, *O, O*-diethyl *O-p*-nitrophenyl phosphorothioate, and it was later confirmed by GC-MS to be ethyl parathion. The other large peak could not be matched with any of the common organophosphorus pesticide standards. The alkyl phosphates found in the blood may have resulted from chemical hydrolysis after death, or from enzyme hydrolysis before death. In

either situation, it may be noted that the total amount of *O, O*-diethyl phosphate and *O, O*-diethyl phosphorothiolate was equivalent to more than 1 ppm of ethyl parathion in the blood. Without the alkyl phosphate analysis the total amount of parathion indicated would probably not have been sufficient to cause death.

In Patient IV, a wide variety of samples was submitted. The toxicant was found to be an 8 : 1 mixture of *O, O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate, or chlorpyrifos, and diethyl mercaptosuccinate *S*-ester with *O, O*-dimethyl phosphorodithioate, or malathion. As in Patient III, the whole blood again showed only about 5% of the toxicant still intact.

Another interesting point that warrants further study is the high level of chlorpyrifos found in the brain of Patient IV. Consequently, a rat-feeding study is being conducted to investigate these levels.

In Patient IV, in the liver sample submitted were two compounds that were extracted by acetone. The earlier eluting component on the GC was identified as chlorpyrifos. A mass spectrum of the second compound indicated a dechlorinated metabolite of chlorpyrifos.⁷ All four of the alkyl metabolites that would be expected from exposure to a mixture of chlorpyrifos and malathion were found in the urine. The phenol metabolite of chlorpyrifos, 2,3,5-

Poison Incidents										
Samples	Intact OP* found	Intact OP	Level of Pesticide or Metabolites, in ppm					Phenol	Remarks	
			DMP†	DEP ‡	DMPTh §	DEPTh				
Patient I										
Urine	NA#	NA	5	<0.05	NA	NA	NA	NA	Confirmed by GC-MS	
Poison Formulation	Dicrotophos									
Patient II										
Blood	Unidentified		38	<0.05	<0.1	<0.1	NA		Confirmed by GC-MS	
Liver	Unidentified		60	<0.05	<0.1	<0.1	NA			
Stomach Contents			450	<0.05	<0.1	<0.1	<0.01			
Patient III										
Blood	Ethyl parathion Malathion Ronnel ronnoxon	0.034 TR** TR	<0.05	0.32	<0.1	0.26	NA		Confirmed by GC-MS Not confirmed Not confirmed	
Patient IV										
Blood	Chlorpyrifos	0.210	0.07	0.32	0.13	0.46	NA		Confirmed by GC-MS Confirmed by GC-MS	
Brain	Chlorpyrifos	0.47	<0.05	0.154	<0.1	<0.1	NA			
Liver	Chlorpyrifos	0.08	ND††	1.58	ND	ND	NA			
Urine	NA	NA	4.04	24.5	7.46	23.6	13.8		Confirmed by GC-MS Confirmed by GC-MS	
Formulation	Chlorpyrifos	8000 (0.8%)	Checked for alkyl phosphates and found very little breakdown							
	Malathion	1000 (0.1%)								
* OP, organophosphorus † Dimethyl phosphate ‡ Diethyl phosphate § Dimethyl phosphorothiolate Diethyl phosphorothiolate # NA, not analyzed ** TR, trace †† ND, non-detectable										

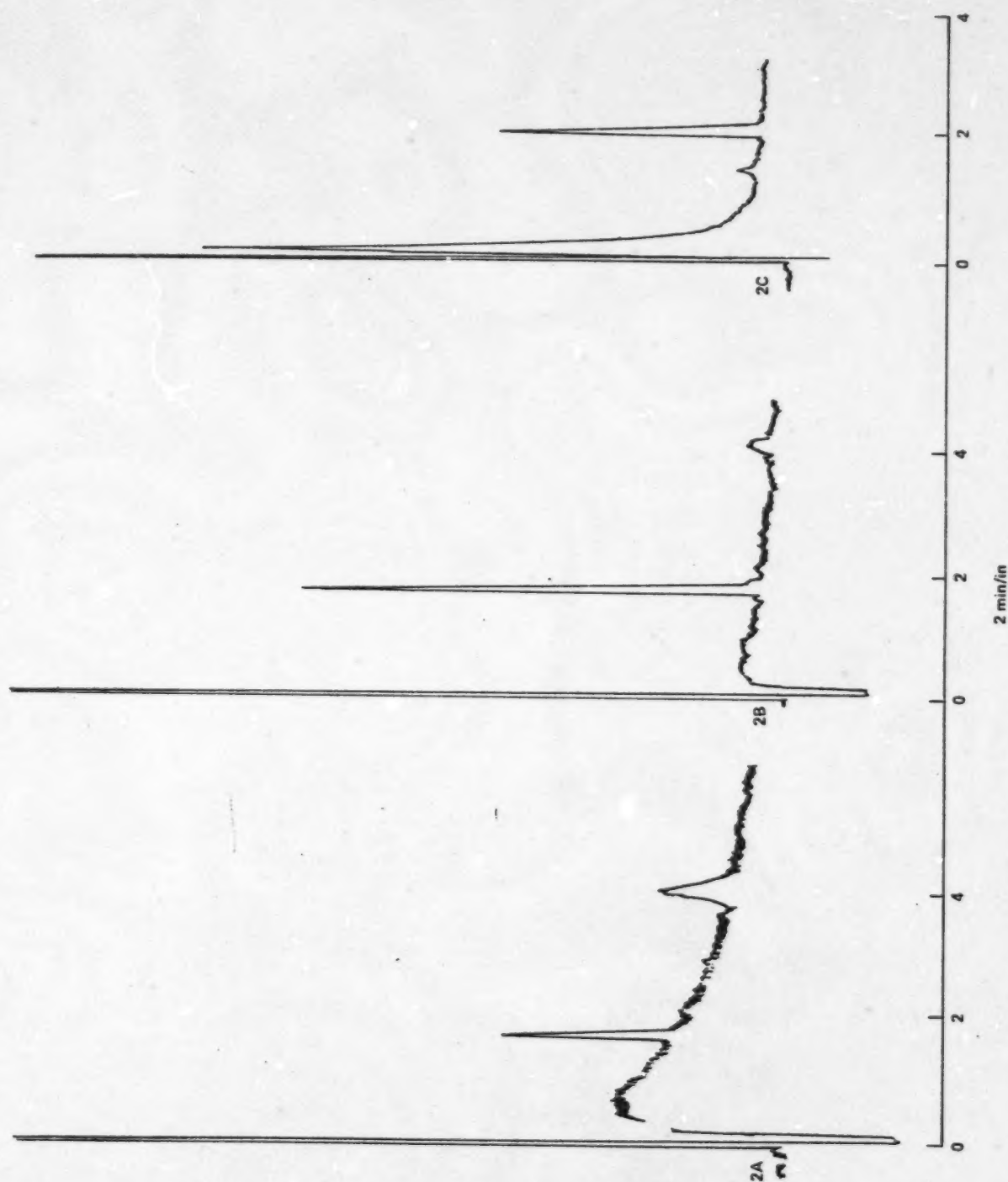


Fig. 1. Chromatograms of acetone extracts of tissues. A), Acetone extract of whole blood from Patient IV. B), Acetone extract of brain from Patient IV. C), Acetone extract of liver from Patient II. All chromatograms were run on a 5% OV 210 column at 180°C.

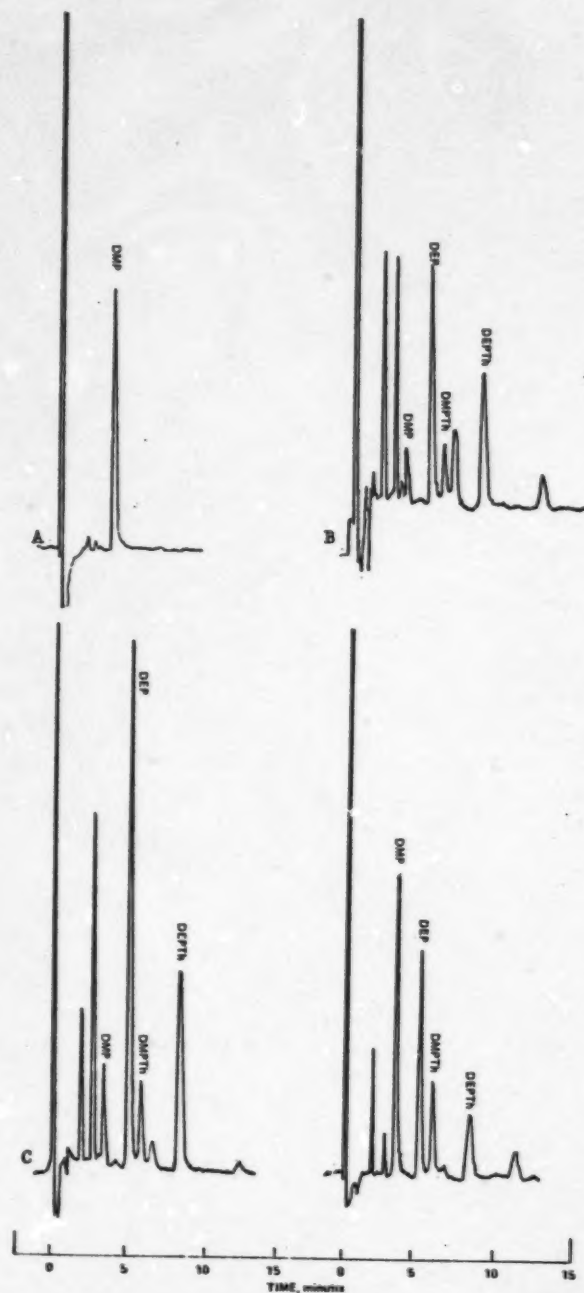


Fig. 2. Chromatograms from alkyl phosphate analysis. A), from alkyl phosphate analysis of liver in Patient II. B), from alkyl phosphate analysis of blood in Patient IV. C), from alkyl phosphate analysis of urine in Patient IV. D), a chromatogram of alkyl phosphate standards. All chromatograms were run on 5% OV 210 column at 140°C.

trichloropyridinol, was also found in the urine. To confirm the presence of chlorpyrifos in these tissues, mass spectra were obtained for both brain and liver extracts.

Chromatograms of some of the tissue extracts are shown in Figure 1. As can be seen, there is almost no interference from the tissues. Chromatograms from the alkyl phosphate analysis in tissues are shown in Figure 2. The interference seen in these chromatograms comes from the reagents.

Analysis of blood, brain, liver, stomach contents, and urine have been demonstrated. The application of this procedure to any of the biological samples available in a poisoning incident should provide a rapid means of identification of suspected organophosphorus toxicants, so that a preliminary identification of the common organophosphorus pesticides can be established in less than 30 minutes. The identification of the type of organophosphorus pesticide can help determine the proper course of treatment for patients suffering from this type of poisoning.

In all samples, except those of brain tissue, the alkyl phosphate analysis was a more sensitive indicator of exposure than the analysis for intact pesticides. A measurement of the intact pesticide combined with the alkyl phosphate analysis gave a better indication of the type and level of exposure, however.

* * * * *

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